myocardial IPC. Furthermore, inhibition of GSNO-reductase has also been shown to increase protein SNO and is cardioprotective. SNO is thought to provide cardioprotection, in part, by modulating enzyme activity. SNO may also provide cardioprotection by reducing cysteine oxidation during ischemia/reperfusion (IR) injury. In order to test the hypothesis that SNO provides cardioprotection by providing direct protection against cysteine oxidation following IR injury, we developed a method to measure protein oxidation using resin-assisted capture (Ox-RAC). This method is similar to the SNO-RAC method used in the quantification of SNO. Langendorff perfused hearts were subjected to various perfusion protocols (control, IPC, IR, IPC-IR) and homogenized. Each sample was divided into two equal aliquots, and subjected to the SNO-RAC/Ox-RAC procedure in order to simultaneously analyze SNO and oxidation. Using the SNO-RAC protocol, we identified 44 different proteins that were SNO with IPC. With the Ox-RAC protocol, we identified nearly 200 oxidized proteins following IR injury. Interestingly, IPC significantly reduced protein oxidation by more than 30% following ischemia and early reperfusion as determined via label free peptide analysis. Additionally, more than half of the proteins which showed significantly increased SNO with IPC, also showed a significant decrease in cysteine oxidation following IPC-IR at the same site when compared to IR alone. These results support the hypothesis that SNO provides cardioprotection by shielding cysteine residues from ROS-induced oxidation during IR injury. Therefore, the level of protein SNO plays a critical role in IR injury, where ROS production is increased.

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Role of Inorganic Polyphosphate for Cardiac Mitochondrial Function in Ischemia/Reperfusion

Lea K. Seidlmayer, Lothar A. Blatter, Evgeny Pavlov, Elena N. Dedkova.

Loss of mitochondrial function plays a critical role in the process of cardiac cell death during ischemia/reperfusion (I/R) injury. Mitochondria contribute to cell death by a combination of several factors that include calcium overload, increased ROS generation, permeability transition pore (mPTP) opening and disruption of energy metabolism. Mitochondrial inorganic polyphosphate (polyP) levels have been implicated to contribute to the regulation of mPTP. Thus, the aim of this study was to investigate the role of polyP in mitochondrial function under conditions of I/R. PolyP is a linear inorganic polymer of many orthophosphates linked together by chemical bonds similar to ATP. In mammalian mitochondria polyP is present in chain length of 60 to 100 orthophosphates. In cultured rabbit ventricular myocytes polyP levels were decreased by adenoviral expression of a mitochondrially targeted polyP hydrolyzing enzyme (PPX). I/R was induced by exposing cells to glucose-free Tyrode solution containing 20 mM 2-deoxyglucose and 2 mM NaCN, pH 6.4, followed by superfusion with standard Tyrode solution. PPX expressing cells with depleted polyP levels (PPX cells) exhibited reduced mPTP opening following I/R measured at the onset of reperfusion as decreased loss of mitochondrially entrapped calcein red (ctrl: $-25 \pm 1\%$, n=20; PPX: $-16 \pm 3\%$, n=14; $p<0.01$). Mitochondrial membrane potential measurements using the potential sensitive dye TMRM showed that polyP depletion lessened mitochondrial membrane potential depolarization induced by I/R (ctrl: $-71 \pm 3\%$, n=19; PPX: $-38 \pm 10\%$, n=15; $p<0.05$). The mPTP blocker Cyclosporin A (CsA) more efficiently prevented mitochondrial membrane potential depolarization in control compared to PPX cells, but did not provide additional protection in PPX cells. We conclude that polyP plays a critical role in regulation of mitochondrial function during I/R. Specifically, our data suggest that polyP contributes towards activation of I/R-induced mPTP opening at early stages of reperfusion.

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Cardioprotection in Brown Norway Rats is Linked to Mitochondrial Complex I Preservation

Raha Nabbi, Ashish K. Gadicherla, Judy R. Kersten, David F. Stowe, Jozef Lazar, Matthias L. Riess.

Hearts from Brown Norway (BN) rats are more resistant to ischemia-reperfusion (IR) injury than hearts from Dahl Salt Sensitive (SS) rats. Moreover, introgression of BN chromosome 6 into SS confers IR resistance to the same degree as BN. We hypothesize that mitochondrial complex I preservation is linked to cardioprotection in this consomic rat model.

Langendorff-prepared hearts from eight week old male BN, SS6BN and SS rats were subjected to 20 min perfusion, 30 min global ischemia or no ischemia (control), and 30 min reperfusion. Mitochondria were isolated and mitochondrial complex I preservation was measured by Western blotting using complex I NDUFA9 subunit antibody.

We found higher protein levels in ischemic BN and SS6BN rat mitochondria compared to SS suggesting better preservation of complex I in BN and SS6BN. In contrast, the non-ischemic groups showed no detectable difference in protein levels.

Previous studies have shown that mitochondrial function can be a trigger as well as an effector of cardioprotection. Our data shows that mitochondrial complex I is better preserved in BN and SS6BN than in SS. Since myocardial and mitochondrial functions differ significantly between the more IR-susceptible SS and the more IR-resistant BN and SS6BN strains, this suggests that rat chromosome 6 encodes one or more genes that are critical for mitochondrial function and cardioprotection.

234-Pos Board B34

The Cardioprotective Effects of Temperature Preconditioning on Isolated Ventricular Myocytes

Yusuf Bhagatte, nina storey.

In *ex vivo* whole heart studies temperature preconditioning (TP) has been found to confer cardioprotection (Khaliulin et al. 2007). Here we have investigated the molecular effects of TP in freshly isolated adult rat cardiac myocytes.

To assess the affect of TP we measured; contractile function, Ca^{2+} homeostasis and mitochondrial permeability transition pore (MPTP) opening. TP consisted of three cycles of 2 minutes at 12°C and 3 minutes at 37°C. Contraction of myocytes was synchronized by electrical field stimulation (1Hz) and recovery of contractile function was measured following simulated ischaemia/reperfusion injury. This consisted of 7 minutes perfusion with substrate-free Tyrode solution containing cyanide (2mM) and iodoacetic acid (1mM) followed by 10 minutes reperfusion with Tyrode solution. A significant increase in contractile function after TP ($52\% \pm 6$ n=225, 3) was observed compared to control ($29\% \pm 5$ n=229, 3) ($p<0.05$). To identify whether this protection was linked to changes in Ca^{2+} handling, we analysed Ca^{2+} levels at 10 minutes reperfusion. We found that TP myocytes had significantly lower Ca^{2+} levels (131nM n=67, 3) compared to controls (301nM n=69, 3) ($p<0.005$). MPTP opening is thought to be a critical event in the determination of myocyte fate following ischaemia/reperfusion injury. Therefore we investigated the effects of TP on the time to induce MPTP opening in response to photodamage using TMRM, a mitochondrial selective dye. We found that TP myocytes significantly delayed time to MPTP opening (15.2 ± 0.6 n=126, 3) compared to controls (11.9 ± 0.4 n=168, 3) ($p<0.0001$).

TP confers protection to isolated myocytes by significantly increasing recovery of contractile function and preventing calcium overload in response to simulated ischaemia/reperfusion. TP also significantly delays MPTP opening after simulated ischaemia/reperfusion injury.

Data presented as mean ± SEM, n=myocytes, animals.

Khaliulin *et al., J physio* 581.3 (2007) 1147-1161.

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235-Pos Board B35

Imaging Intracellular and Intra-Mitochondrial Free Zinc Ion Concentrations Following Hypoxia/Hypoglycemia with an Expressible Fluorescence Biosensor

Bryan McCranor, Linda Bambrick, Brian Polster, Rebecca A. Bozym, Michele Vitolo, Gary Fiskum, Carol A. Fierke, Richard B. Thompson.

Zinc is a ''trace'' metal necessary for cellular function, but excess free zinc ion is toxic to most cells (1,2,9) We and others have observed increased intra- and extra-cellular free zinc concentrations following cerebral ischemia (3,4). Substantial evidence indicates that mitochondrial dysfunction plays a significant role in neuronal death following ischemia (5), and both mitochondrial dysfunction and increased intracellular zinc have been associated with increased production of reactive oxygen species (ROS) and ultimately apoptosis (6,7). Zinc, potently, inhibits mitochondrial enzymes involved in energy production and destruction of ROS, potentially promoting mitochondrial dysfunction (8). We targeted our expressible fluorescent zinc biosensor (9) to mitochondria of PC12 cells, to ratiometrically image the intra-mitochondrial zinc concentration at resting (pM) levels. We imaged cytoplasmic and mitochondrial zinc levels in cells deprived of oxygen and glucose (OGD), a widely used model of ischemia. Our data indicate that both intra-mitochondrial and cytoplasmic zinc concentrations increase substantially following OGD. Further experiments indicate that the zinc is released from intracellular sites rather than entering the cell from external sources. Supported by NIH EB03924.

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